

AD \_\_\_\_\_

Award Number: DAMD17-01-1-0481

TITLE: The Structural Basis for the Role of CHK as a Tumor  
Suppressor Protein in Human Breast Cancer

PRINCIPAL INVESTIGATOR: Jerome E. Groopman, M.D.  
Gordon D. Webster, Ph.D.

CONTRACTING ORGANIZATION: Beth Israel Deaconess Medical Center  
Boston, Massachusetts 02215-5399

REPORT DATE: May 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20030313 122

# REPORT DOCUMENTATION PAGE

Form Approved  
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE May 2002	3. REPORT TYPE AND DATES COVERED Annual (1 May 01 - 30 Apr 02)	
4. TITLE AND SUBTITLE  The Structural Basis for the Role of CHK as a Tumor Suppressor Protein in Human Breast Cancer			5. FUNDING NUMBERS DAMD17-01-1-0481	
6. AUTHOR(S) Jerome E. Groopman, M.D. Gordon D. Webster, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)  Beth Israel Deaconess Medical Center Boston, Massachusetts 02215-5399 E-Mail :jgroopma@caregroup.harvard.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)  U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT  Approved for Public Release; Distribution Unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 Words)  The goal of this project is to determine how CHK functions at the molecular level, crystals of the closed and purified, recombinant CHK SH2 domain will be obtained for X-ray diffraction studies from which we can determine its precise, three-dimensional, molecular structure. Our research will focus upon the structural details of how CHK recognizes the tail of the active Her-2/neu receptor.				
14. SUBJECT TERMS CHK, breast cancer, Her-2/new receptor			15. NUMBER OF PAGES 4	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)  
Prescribed by ANSI Std. Z39-18  
298-102

## Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4
Reportable Outcomes.....	4
Appendices.....	4

### **Introduction:**

We have made considerable progress towards the goals of the project. This work set the stage for developing structural information that may assist in designing novel therapeutics against breast cancer based on mimicking the activity of CHK as an inhibitor of Her2/neu pathway.

### **Body:**

Experiments were conducted as described in the original proposal. In addition, advances were made using different expression systems to obtain purified CHK proteins for structural analysis.

### **Key Research Accomplishments:**

Over the past year, we successfully expressed different truncated variants of SH2 domain of human CHK from the pGEX vector in *E. coli*. Each of the variants was purified by affinity chromatography and subjected to preliminary crystallization trials using hampton screens. One of the variants yielded preliminary crystals which diffracted to a resolution of 2.5 Å and extensive optimization yielded crystals which diffracted to a resolution of 1.8 Å. The crystal structure of the SH2 domain of CHK was solved at a resolution of 2.5 Å by molecular replacement method and deposited in the data bank (PDB ID: 1JWO).

Unfortunately, attempts to co-crystallize the SH2 domain with several synthetic peptide analogs of HER2/neu receptor did not yield diffractable crystals.

The crystal structure of the SH2 domain was modeled with a peptide using energy minimization. In addition, the kinase domain of CHK was expressed and affinity purified to ~1mg per liter culture from *Pichia pastoris*. To date, we are unable to express full length and some truncated variants of CHK from *Pichia pastoris*.

The SH2 domains of CHK and Csk share ~80% sequence homology. Comparison of the structure of SH2 domain of CHK with that of Csk could yield useful information with regards to the interaction with the HER2/neu receptor. To that end, we cloned and expressed from the pGEX vector, in *E. coli*, the SH2 domains of CHK.

Thus, we have made considerable progress towards our goal but have not yet achieved the ultimate objective of a co-crystal of CHK with the Her2/neu receptor. Our efforts continue using parallel strategies to overcome the pitfalls we face in certain expression systems and feel that these new avenues may soon be fruitful.

**Reportable Outcomes:** We plan to report the methods for crystallization of CHK SH2 domain and have deposited in the databank the crystal structure of the CHK SH2 domain with a resolution of 2.5 Å.

### **Conclusions:**

CHK is an important regulator of breast cancer growth and spread and appears to act by interaction with the Her2/neu receptor. Our work has advanced to the field in obtaining some structural information on CHK and set the stage for further studies that could lead to novel therapies against breast cancer.

**References:** none

**Appendices:** none